H24 A Metabolomic Profile of Aqueous Humor in a 24-Hour Period After Death: An Animal Model for Postmortem Interval (PMI) Estimation

Emanuela Locci, PhD, Cagliari, ITALY; Matteo Stocchero, PhD, Padova, ITALY; Alberto Chighine, MD, Cagliari, ITALY; Fabio De Giorgio, MD, Catholic University of the Sacred Heart, Rome 00168, ITALY; Matteo Nioi, MD, Cagliari, ITALY; Ernesto d'Aloja, MD, PhD, Rome, ITALY*

Learning Overview: After attending this presentation, attendees will understand the potential role of metabolomics in estimating PMI through the study of aqueous humor.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing one more tool in the investigation of PMI.

The estimation of the time since death or PMI remains a major challenge in forensic pathology. Several forensic laboratories are involved in ongoing efforts to characterize and validate an objective analytical method for this purpose. Besides traditional methods based on macroscopic corpse modification, different analytical procedures have been recently proposed for this aim. These include the evaluation of messenger RNA (mRNA) or DNA degradation in postmortem tissues, human tissue transcriptomes, and gene expression patterns, muscle protein degradation based on protein or peptide signatures, the estimation of the hypoxic inducible levels of vascular endothelial growth factor, and assessing postmortem biochemical changes in body fluids (e.g., blood, synovial, pericardial, and cerebrospinal fluids.) Ocular tissues and fluids—especially vitreous humor— have been frequently chosen as reference biological samples due to being more anatomically isolated. Recently, metabolomics have shown to be a potential tool to investigate the time-related postmortem metabolite modifications in animal models. While traditional techniques for PMI estimation are quite subjective in nature, and other proposed methodologies are based on the estimation over time of a single to a few parameters that are potentially prone to the influence of intrinsic/extrinsic factors, the analysis of metabolomic modifications, relying on multiple metabolites/biomarkers quali-quantitative changes, shows a greater predictive power. Here is proposed, for the first time, the use of a ^{1}H NMR metabolomic approach for the estimation of PMI from Aqueous Humor (AH) in an ovine model.

A total of 59 AH samples were collected at different PMI (spanning from 118 up to 1,429 minutes, at a 60-minute pace). Thirty-eight (38/59) were used for the training set, while the remaining 21 were employed as test set. ^{1}H NMR experiments were performed, and the spectral data was analyzed by multivariate statistical tools. Exploratory data analysis was performed by Principal Component Analysis (PCA) to discover outliers and specific trends in the data. Thus, supervised data analysis based on Projection to Latent Structure regression (PLS2) was applied to evaluate the effects of PMI on the metabolomic profiles of the collected samples. A multivariate calibration model was built to estimate PMI on the basis of the metabolite content of the samples. The model was validated with an independent test set, obtaining a prediction error of 59 minutes for PMI less than 500 minutes, 104 minutes for PMI from 500 to 1,000 minutes, and 118 minutes for PMI greater than 1,000 minutes. During the first 1,000 minutes, lactate was accumulated and strongly influenced the sample distribution along PC1. Early PMI samples were characterized by high levels of leucine and isoleucine, arginine, and lysine, while late PMI samples (>1,000 minutes) showed high levels of taurine, succinate, and choline. Moreover, the metabolomic approach suggested a picture of the mechanisms underlying the postmortem biological modifications, highlighting the role played by taurine, choline, and succinate. The time-related modifications of the ^{1}H NMR AH metabolomic profile in the first 24 hours after death seem to be encouraging in addressing the issue of a reproducible and robust model to be employed for the estimation of PMI.

Metabolomics, Aqueous Humor, PMI Estimation

*Presenting Author